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⑲ Liquid detergents containing surfactant, proteolytic enzyme and boric acid.

⑳ Liquid detergent compositions containing synthetic surfactant, specific proteolytic enzyme, boric acid or a boron compound capable of forming boric acid in the composition, and calcium ion are disclosed. The compositions exhibit improved enzyme stability because boric acid stabilizes the proteases herein to a greater degree than it does other proteases. Preferred laundry detergent compositions containing anionic surfactant and detergency builder also provide improved cleaning performance, particularly through-the-wash, on enzyme-sensitive stains.

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## LIQUID DETERGENTS CONTAINING SURFACTANT, PROTEOLYTIC ENZYME AND BORIC ACID

## Technical Field

The present invention relates to liquid detergent compositions containing synthetic surfactant, specific proteolytic enzyme, boric acid or a boron compound capable of forming boric acid in the composition, and calcium ion. The compositions exhibit improved enzyme stability because boric acid stabilizes the proteases herein to a greater degree than it does other proteases. The compositions are preferably heavy-duty liquid laundry detergents, but can also be light-duty liquid detergents suitable for dishwashing or washing fine fabrics, detergent pretreatment compositions or all-purpose household liquid cleaners. Preferred laundry detergent compositions containing a relatively high level of anionic surfactant and detergency builder also provide improved cleaning performance, particularly through-the-wash, of enzyme-sensitive stains such as grass, blood, gravy and chocolate pudding.

Laundry detergents containing high levels of anionic surfactant and builder, and capable of providing superior cleaning performance, are currently available. Some of these compositions also contain enzymes to enhance removal of enzyme-sensitive stains. However, it is believed that such compositions are enzyme-limited in that they can denature and expose stains to enzymatic action faster than currently available enzymes can cleave and break up the stains.

Enzyme performance can also be limited by a lack of adequate stability in liquid detergents. The stabilization of enzymes is particularly difficult in built, heavy-duty liquid detergents containing high levels of anionic surfactant and water. Anionic surfactants, especially alkyl sulfates, tend to denature enzymes and render them inactive. Detergent builders can sequester the calcium ion needed for enzyme activity and/or stability.

Thus, there is a continuing need for the development of new enzymes that provide improved performance and better stability in liquid detergent compositions, particularly those containing high levels of anionic surfactant and builder.

## Background Art

U.S. Patent 4,261,868, Hora et al, issued April 14, 1981, discloses liquid detergents containing enzymes and, as an enzyme-stabilizing system, 2-25% of a polyfunctional amino compound selected

from diethanolamine, triethanolamine, diisopropanolamine, triisopropanolamine and tris(hydroxymethyl) aminomethane, and 0.25-15% of a boron compound selected from boric acid, boric oxide, borax, and sodium ortho-, meta-and pyroborate. The compositions can contain 10-60% surfactant, including anionics, and up to 40% builder.

U.S. Patent 4,404,115, Tai, issued September 13, 1983, discloses liquid cleaning compositions, preferably built liquid detergents, containing enzyme, 1-15% alkali metal pentaborate, 0-15% alkali metal sulfite, and 0-15% of a polyol having 2-6 hydroxy groups. The compositions can contain 1-60% surfactant, preferably a mixture of anionic and nonionic in a weight ratio of 6:1 to 1:1, with or without soap. The compositions also preferably contain 5-50% builder.

U.S. Patent 4,318,818, Letton et al, issued March 9, 1982, discloses liquid detergents containing enzymes and an enzyme-stabilizing system comprising calcium ion and a low molecular weight carboxylic acid or salt, preferably a formate. The compositions preferably contain from about 20% to 50% surfactant, which can be anionic. In a preferred embodiment, the compositions contain about 3% to 15% of a saturated fatty acid. They are otherwise substantially free of builders, but can contain minor amounts of sequestrants.

European Patent Application 130,756, published January 9, 1985, discloses the proteolytic enzymes herein and methods for their preparation. The enzymes are said to be useful in laundry detergents, both liquid and granular. They can be combined with surfactants (including anionic), builders, bleach and/or fluorescent whitening agents, but there is no disclosure of specific detergent compositions.

## 40 Summary of the Invention

This invention relates to liquid detergent compositions comprising, by weight:

- 45 (a) from about 1% to about 75% of a synthetic detergent surfactant;
- 50 (b) from about 0.01% to about 5% of the proteolytic enzyme characterized by the following amino acid sequence:

1 10  
Ala Gln Ser Val Pro Tyr Gly Val Ser Gln Ile Lys Ala Pro  
20  
Ala Leu His Ser Gln Gly Tyr Thr Gly Ser Asn Val Lys Val  
30 40  
Ala Val Ile Asp Ser Gly Ile Asp Ser Ser His Pro Asp Leu  
50  
Lys Val Ala Gly Gly Ala Ser Met Val Pro Ser Glu Thr Asn  
60 70  
Pro Phe Gln Asp Asn Asn Ser His Gly Thr His Val Ala Gly  
80  
Thr Val Ala Ala Leu Asn Asn Ser Ile Gly Val Leu Gly Val  
90  
Ala Pro Ser Ala Ser Leu Tyr Ala Val Lys Val Leu Gly Ala  
100 110  
Asp Gly Ser Gly Gln Tyr Ser Trp Ile Ile Asn Gly Ile Glu

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120

Trp Ala Ile Ala Asn Asn Met Asp Val Ile Asn Met Ser Leu  
 130 140

Gly Gly Pro Ser Gly Ser Ala Ala Leu Lys Ala Ala Val Asp  
 150

Lys Ala Val Ala Ser Gly Val Val Val Val Ala Ala Ala Gly  
 160

Asn Glu Gly Thr Ser Gly Ser Ser Ser Thr Val Gly Tyr Pro  
 170 180

Gly Lys Tyr Pro Ser Val Ile Ala Val Gly Ala Val Asp Ser  
 190

Ser Asn Gln Arg Ala Ser Phe Ser Ser Val Gly Pro Glu Leu  
 200 210

Asp Val Met Ala Pro Gly Val Ser Ile Gln Ser Thr Leu Pro  
 220

Gly Asn Lys Tyr Gly Ala Tyr Asn Gly Thr Ser Met Ala Ser  
 230

Pro His Val Ala Gly Ala Ala Ala Leu Ile Leu Ser Lys His  
 240 250

Pro Asn Trp Thr Asn Thr Gln Val Arg Ser Ser Leu Glu Asn  
 260

Thr Thr Thr Lys Leu Gly Asp Ser Phe Tyr Tyr Gly Lys Gly  
 270 275

Leu Ile Asn Val Gln Ala Ala Ala Gln;

(hereinafter referred to as Protease A); or  
 wherein the Gly at position 166 is replaced  
 with Ser or Asn, the Gly at position 169 is  
 replaced with Ser, or the Met at position 222  
 is replaced with Phe;

(c) from about 0.1% to about 10% of boric  
 acid or a boron compound capable of for-  
 ming boric acid in the composition;

(d) from about 0.01 to about 50 millimoles of  
 calcium ion per liter of composition; and

(e) from about 10% to about 95% of water;

#### Detailed Description of the Invention

The liquid detergents of the present invention  
 contain, as essential components, synthetic deter-  
 gent surfactant, specific proteolytic enzyme, boric  
 acid or a boron compound capable of forming boric  
 acid in the composition, calcium ion, and water.  
 The compositions exhibit improved enzyme stabil-  
 ity because boric acid stabilizes the proteases  
 herein to a greater degree than it does other pro-  
 teases. This is particularly surprising given that the  
 proteases herein do not exhibit improved stability  
 in the absence of boric acid.

While not intending to be limited by theory, it  
 is believed that boric acid and calcium form in-  
 tramolecular bonds which cross-link or staple the  
 enzyme molecule together, thereby holding it in its  
 active spatial conformation. This mechanism is ap-  
 parently more effective for the proteases herein  
 than for other proteases.

It is also believed that the relatively high level of anionic surfactant and builder in the preferred compositions herein provides an effective matrix for denaturing stains and exposing sites to enzymatic action. The anionic surfactant is believed to be the primary denaturing agent, whereas the builder controls water hardness that would otherwise complex the anionic surfactant and interfere with its denaturing action. Once the stains are denatured, enzymes bind to the exposed sites and clip chemical bonds before returning to solution to begin the cycle again. After a sufficient number of clips are made, the stained fragments are removed and/or solubilized by the surfactants. However, it is believed that the preferred surfactant and builder matrix herein can denature and expose more sites on stains than currently available enzymes can cleave during the washing process. This is particularly true at low washing temperatures (e.g., in the range of 15°C to 35°C) where enzymes are catalytically slow. The present proteolytic enzymes appear to be superior to other proteases in catalytic efficiency. They thus can take advantage of the stain denaturing power of the preferred compositions herein and provide significant stain removal benefits. In contrast, they provide little or no stain removal benefits in detergent compositions containing less anionic surfactant and builder.

#### Synthetic Surfactant

The compositions of the present invention contain from about 1% to about 75%, preferably from about 5% to about 50%, and most preferably from about 15% to about 35%, by weight of a synthetic surfactant, which can be an anionic, non-ionic, cationic, zwitterionic or amphotolytic surfactant, or mixtures thereof. Suitable synthetic surfactants are disclosed in U.S. Patent 4,285,841, Barrat et al, issued August 25, 1981, and in U.S. Patent 3,929,678, Laughlin et al, issued December 30, 1975, both incorporated herein by reference.

Preferred compositions herein contain from about 7% to about 50%, preferably from about 10% to about 40%, more preferably from about 15% to about 30%, by weight of an anionic synthetic surfactant.

Useful anionic surfactants include the water-soluble salts, particularly the alkali metal, ammonium and alkylolammonium (e.g., monoethanolammonium or triethanolammonium) salts, of organic sulfuric reaction products having in their molecular structure an alkyl group containing from about 10 to about 20 carbon atoms and a sulfonic acid or sulfuric acid ester group. (Included in the term "alkyl" is the alkyl portion of aryl groups.) Exam-

ples of this group of synthetic surfactants are the alkyl sulfates, especially those obtained by sulfating the higher alcohols ( $C_8$ - $C_{18}$  carbon atoms) such as those produced by reducing the glycerides of tallow or coconut oil; and the alkylbenzene sulfonates in which the alkyl group contains from about 9 to about 15 carbon atoms, in straight chain or branched chain configuration, e.g., those of the type described in U. S. Patents 2,220,099 and 2,477,383. Especially valuable are linear straight-chain alkylbenzene sulfonates in which the average number of carbon atoms in the alkyl group is from about 11 to 14.

Other anionic surfactants herein are the water-soluble salts of: paraffin sulfonates containing from about 8 to about 24 (preferably about 12 to 18) carbon atoms; alkyl glyceryl ether sulfonates, especially those ethers of  $C_{8-18}$  alcohols (e.g., those derived from tallow and coconut oil); alkyl phenol ethylene oxide ether sulfates containing from about 1 to about 4 units of ethylene oxide per molecule and from about 8 to about 12 carbon atoms in the alkyl group; and alkyl ethylene oxide ether sulfates containing about 1 to about 4 units of ethylene oxide per molecule and from about 10 to about 20 carbon atoms in the alkyl group.

Other useful anionic surfactants include the water-soluble salts of esters of alpha-sulfonated fatty acids containing from about 6 to 20 carbon atoms in the fatty acid group and from about 1 to 10 carbon atoms in the ester group; water-soluble salts of 2-acyloxy-alkane-1-sulfonic acids containing from about 2 to 9 carbon atoms in the acyl group and from about 9 to about 23 carbon atoms in the alkane moiety; water-soluble salts of olefin sulfonates containing from about 12 to 24 carbon atoms; and beta-alkyloxy alkane sulfonates containing from about 1 to 3 carbon atoms in the alkyl group and from about 8 to 20 carbon atoms in the alkane moiety.

Preferred anionic surfactants are the  $C_{10}$ - $C_{18}$  alkyl sulfates and alkyl ethoxy sulfates containing an average of up to about 4 ethylene oxide units per mole of alkyl sulfate,  $C_{10}$ - $C_{18}$  linear alkylbenzene sulfonates, and mixtures thereof.

The compositions preferably contain from about 1% to about 5%, more preferably from about 2% to about 4%, by weight of unethoxylated alkyl sulfate. These alkyl sulfates are desired for best detergency performance, in part because they are very denaturing to stains.

A preferred cosurfactant, used at a level of from about 1% to about 25%, preferably from about 3% to about 15%, by weight of the composition, is an ethoxylated nonionic surfactant of the formula  $R'(OC_2H_4)_nOH$ , wherein  $R'$  is a  $C_{10}$ - $C_{16}$  alkyl

group or a  $C_6$ - $C_{12}$  alkyl phenyl group, n is from about 3 to about 9, and said nonionic surfactant has an HLB (hydrophile-lipophile balance) of from about 6 to about 14, preferably from about 10 to about 13. These surfactants are more fully described in U.S. Patents 4,285,841, Barrat et al, issued August 25, 1981, and 4,284,532, Leikhim et al, issued August 18, 1981, both incorporated herein.



wherein  $\text{R}^1$  is an alkyl, hydroxyalkyl or alkenyl radical containing from about 8 to about 20 carbon atoms, and  $\text{R}^2$  and  $\text{R}^3$  are selected from the group consisting of hydrogen, methyl, ethyl, propyl, isopropyl, 2-hydroxyethyl, 2-hydroxypropyl, 3-hydroxypropyl, and said radicals additionally containing up to about 5 ethylene oxide units, provided at least one of  $\text{R}^2$  and  $\text{R}^3$  contains a hydroxyl group.

Preferred amides are the  $C_8$ - $C_{20}$  fatty acid alkylol amides in which each alkylol group contains from 1 to 3 carbon atoms, and additionally can contain up to about 2 ethylene oxide units. Particularly preferred are the  $C_{12}$ - $C_{16}$  fatty acid monoethanol and diethanol amides.

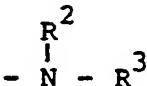
Certain compositions herein preferably contain from about 5% to about 20%, preferably from about 6% to about 15%, more preferably from about 7% to about 12%, by weight of a mixture of the above ethoxylated nonionic surfactant and amide surfactant in a weight ratio of from about 4:1 to 1:4, preferably from about 3:1 to about 1:3, more preferably from about 2:1 to about 1:2. In addition, the weight ratio of anionic synthetic surfactant (on an acid basis) to the total nonionic surfactant (both the ethoxylated nonionic and the amide) should be from about 2:1 to about 4:1, preferably from about 2.5:1 to about 3.5:1, to ensure the formation and adsorption of sufficient hardness surfactants at the oil/water interface to provide good greasy/oily soil removal.

Other preferred cosurfactants, used at a level of from about 0.5% to about 3%, preferably from about 0.7% to about 2%, by weight are the quaternary ammonium, amine or amine oxide surfactants described in U.S. Patent 4,507,219, Hughes, issued March 26, 1985, incorporated herein by reference.

Where the compositions herein can contain di-long chain quaternary ammonium cationic surfactants (e.g., those having 2 chains, each containing an average of from about 16 to about 22 carbon atoms), such as disclosed in British Patent

in by reference. Particularly preferred are condensation products of  $C_{12}$ - $C_{15}$  alcohols with from about 3 to about 8 moles of ethylene oxide per mole of alcohol, e.g.,  $C_{12}$ - $C_{15}$  alcohol condensed with about 6.5 moles of ethylene oxide per mole of alcohol.

Preferred cosurfactants for use with the above ethoxylated nonionic surfactants are amides of the formula



2,041,968, Murphy, published September 19, 1979, incorporated herein by reference, the compositions preferably contain less than about 2%, more preferably less than about 1%, by weight of such surfactants. Most preferably, the compositions are substantially free of such surfactants because they appear to be detrimental to the stability of the proteolytic enzymes herein.

#### Optional Detergency Builder

The compositions also preferably contain from about 5% to about 40%, more preferably from about 8% to about 30%, most preferably from about 10% to about 25%, by weight of a detergent builder material. In addition, the composition preferably contain at least about 20%, more preferably from about 25% to about 60%, most preferably from about 30% to about 50%, by weight of the anionic synthetic surfactant and builder. Since the proteolytic enzymes herein appear to provide optimum performance benefits versus other enzymes when the builder to water hardness ratio is close to one, the compositions preferably contain sufficient builder to sequester from about 2 to about 10, preferably from about 3 to about 8, grains per gallon of hardness.

Useful builders are fatty acids containing from about 10 to about 22 carbon atoms. Preferred are saturated fatty acids containing from about 10 to about 18, preferably from about 10 to about 14, carbon atoms. When present, the fatty acid preferably represents about 5% to about 20%, more preferably from about 8% to about 16%, by weight of the composition.

Suitable saturated fatty acids can be obtained from natural sources such as plant or animal esters (e.g., palm kernel oil, palm oil and coconut oil) or synthetically prepared (e.g., via the oxidation of petroleum or by hydrogenation of carbon monoxide via the Fisher-Tropsch process). Examples of suitable saturated fatty acids for use in the composi-

tions of this invention include capric, lauric, myristic, coconut and palm kernel fatty acid. Preferred are saturated coconut fatty acids; from about 5:1 to 1:1 (preferably about 3:1) weight ratio mixtures of lauric and myristic acid; mixtures of the above with minor amounts (e.g., 1%-30% of total fatty acid) of oleic acid; and palm kernel fatty acid.

Detergent builders useful herein also include the polycarboxylate, polyphosphonate and polyphosphate builders described in U.S. Patent 4,284,532, Leikhim et al, issued August 18, 1981, incorporated herein by reference. Water-soluble polycarboxylate builders, particularly citrates, are preferred of this group. Polycarboxylate builders preferably represent from about 1% to about 20% by weight of the composition.

Suitable polycarboxylate builders include the various aminopolycarboxylates, cycloalkane, polycarboxylates, ether polycarboxylates, alkyl polycarboxylates, epoxy polycarboxylates, tetrahydrofuran polycarboxylates, benzene polycarboxylates, and polyacetal polycarboxylates.

Examples of such polycarboxylate builders are sodium and potassium ethylenediaminetetraacetate; sodium and potassium nitrilotriacetate; and water-soluble salts of phytic acid, e.g., sodium and potassium phytates, disclosed in U.S. Patent 1,739,942, Eckey, issued March 27, 1956, incorporated herein by reference; the polycarboxylate materials described in U.S. Patent 3,364,103, incorporated herein by reference; and the water-soluble salts of polycarboxylate polymers and copolymers described in U.S. Patent 3,308,067, Diehl, issued March 7, 1967, incorporated herein by reference.

Useful detergent builders also include the water-soluble salts of polymeric aliphatic polycarboxylic acids having the following structural and physical characteristics: (a) a minimum molecular weight of about 350 calculated as to the acid form; (b) an equivalent weight of about 50 to about 80 calculated as to acid form; (3) at least 45 mole percent of the monomeric species having at least two carboxyl radicals separated from each other by not more than two carbon atoms; (d) the site of attachment of the polymer chain of any carboxyl-containing radical being separated by not more than three carbon atoms along the polymer chain from the site of attachment of the next carboxyl-containing radical. Specific examples of such builders are the polymers and copolymers of itaconic acid, aconitic acid, maleic acid, mesaconic acid, fumaric acid, methylene malonic acid, and citraconic acid.

Other suitable polycarboxylate builders include the water-soluble salts, especially the sodium and potassium salts, of mellitic acid, citric acid, pyromellitic acid, benzene pentacarboxylic acid, oxydiacetic acid, carboxymethoxysuccinic acid, carboxymethoxymalonic acid, *cis*-cyclohexanehexacarboxylic acid, *cis*-cyclopentanetetracarboxylic acid and oxydisuccinic acid.

Other polycarboxylates for use herein are the polyacetal carboxylates described in U.S. Patent 4,144,226, issued March 13, 1979 to Crutchfield et al, and U.S. Patent 4,146,495, issued March 27, 1979 to Crutchfield et al, both incorporated herein by reference.

Other detergent builders useful herein include the aluminosilicate ion exchange material described in U.S. Patent 4,405,483, Kuzel et al, issued September 20, 1983, incorporated herein by reference.

As part of the builder system, the compositions herein preferably contain from about 0.1% to about 1%, more preferably from about 0.2% to about 0.6%, by weight of water-soluble salts of ethylenediamine tetramethylenephosphonic acid, diethylenetriamine pentamethylenephosphonic acid, ethylenediamine tetraacetic acid, or diethylenetriamine pentaacetic acid to enhance cleaning performance when pretreating fabrics.

#### Proteolytic Enzyme

The compositions of the present invention contain from about 0.01% to about 5%, preferably from about 0.1% to about 2%, by weight of the composition of Protease A as previously defined, or variants thereof in which the Gly at position 166 is replaced with Ser or Asn, the Gly at position 169 is replaced with Ser, or the Met at position 222 is replaced with Phe.

These proteases, and methods for their preparation, are described in European Patent Application 130,756, published January 9, 1985, incorporated herein by reference.

The above enzyme is preferably included in an amount sufficient to provide an activity of from about 0.001 to about 0.1, more preferably from about 0.005 to about 0.07, most preferably from about 0.01 to about 0.04, Anson units per gram of composition.

The proteases herein are preferably purified, prior to incorporation in the finished composition, so that they have no detectable odor at a concentration of less than about 0.002 Anson units per gram in distilled water. They preferably have no detectable odor at a concentration of less than about 0.0025, more preferably less than about 0.003, Anson units per gram of distilled water.

Proteases herein can be odor purified by any method known in the art. Examples include the solvent precipitation methods described in Precipitation of the Enzymes and Their Stability in High Alcohol Concentrations by Bauer et al in the Israel J. Chem. 5(3), pages 117-20 (1967) and Enzyme Preparations by Sugiura et al and Yakusaigaku 1967, Volume 27(2), pages 135-9.

Solvent initiated precipitation of a crude commercial enzyme solution results in most of the enzymatic activity being precipitated from solution and most of the odor and color impurities remaining in the supernatant liquid. Decantation or centrifugation of the supernatant liquid from the precipitated enzyme results in an enzyme fraction with enriched enzymatic activity/gram and improved odor and color.

Various solvents or solvent pair combinations can be used to effect the desired precipitation. For example, methanol, ethanol, acetone, other organic solvents, and combinations of organic solvents with and without water can be used. A highly preferred solvent is a combination of water and 30-70% by weight ethanol. This appears to be optimal to prevent enzyme deactivation and maximum recovery of activity.

Purification of protease enzymes also provide benefits in the area of product color stability.

#### Boric Acid

The compositions of the present invention also contain from about 0.1% to about 10%, more preferably from about 0.25% to about 5%, most preferably from about 0.5% to about 3%, by weight of boric acid or a compound capable of forming boric acid in the composition (calculated on the basis of the boric acid). Boric acid is preferred, although other compounds such as boric oxide, borax and other alkali metal borates (e.g., sodium ortho-, meta- and pyroborate, and sodium pentaborate) are suitable. Substituted boric acids (e.g., phenylboronic acid, butane boronic acid, and p-bromo phenylboronic acid) can also be used in place of boric acid.

#### Calcium Ion

The composition also contains from about 0.01 to about 50, preferably from about 0.1 to about 30, more preferably from about 1 to about 20, millimoles of calcium ion per liter. The level of calcium ion should be selected so that there is always some minimum level available for the enzyme, after allowing for complexation with builders, etc., in the composition. Any water-soluble calcium salt

can be used as the source of calcium ion, including calcium chloride, calcium formate, and calcium acetate. A small amount of calcium ion, generally from about 0.05 to about 0.4 millimoles per liter, is often also present in the composition due to calcium in the enzyme slurry and formula water.

#### Water

Finally, the compositions herein contain from about 10% to about 95%, preferably from about 20% to about 70%, more preferably from about 30% to about 50%, by weight of water.

#### Other Optional Components

Other preferred enzyme stabilizers for use in the present compositions are polyols containing only carbon, hydrogen and oxygen atoms. They preferably contain from 2 to 6 carbon atoms and from 2 to 6 hydroxy groups. Examples include propylene glycol (especially 1,2 propane diol, which is preferred), ethylene glycol, glycerol, sorbitol, mannitol, and glucose. The polyol generally represents from about 1% to about 15%, preferably from about 1.5% to about 10%, by weight of the composition. Preferably, the weight ratio of polyol to boric acid is at least 1, more preferably at least about 1.3.

The compositions can also contain the water-soluble, short chain carboxylates described in U.S. Patent 4,318,818, Letton et al, issued March 9, 1982, incorporated herein by reference. The formates are preferred and can be used at levels of from about 0.05% to about 5%, preferably from about 0.2% to about 2%, most preferably from about 0.4% to about 1.5%, by weight of the composition.

The compositions herein preferably have an initial pH of from about 6.5 to about 10.0, preferably from about 7 to about 8.5, most preferably from about 7.2 to about 8.0, at a concentration of 0.2% by weight in distilled water at 20°C. Preferred pH buffers include monoethanolamine and triethanolamine. Monoethanolamine and triethanolamine also further enhance enzyme stability, and preferably are included at levels of from about 0.5% to about 10%, preferably from about 1% to about 4%, by weight of the composition.

Other optional components for use in the liquid detergents herein include soil removal agents, antire-deposition agents, suds regulators, hydrotropes, opacifiers, antioxidants, bactericides,

dyes, perfumes, and brighteners known in the art. Such optional components generally represent less than about 15%, preferably from about 1% to about 10%, by weight of the composition.

Particularly preferred stable isotropic liquid detergents herein are described in U.S. Patent 4,507,219, Hughes, issued March 26, 1985, incorporated herein by reference.

The following examples illustrate the compositions of the present invention.

All parts, percentages and ratios used herein are by weight unless otherwise specified.

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#### EXAMPLE I

The following detergent compositions were prepared.

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<u>Component</u>	<u>Wt. %</u>				
	<u>A</u>	<u>B</u>	<u>C</u>	<u>D</u>	<u>E</u>
C <sub>13</sub> linear alkylbenzene sulfonic acid	7.2	8.0	-	-	8.0
C <sub>14-15</sub> alkyl polyethoxylate (2.25) sulfuric acid	10.8	12.0	-	-	12.0
C <sub>12-14</sub> alkyl polyethoxylate (1) sulfuric acid (Alkyl sulfuric acid)	-	-	8.8	-	(2.8)
C <sub>12-13</sub> alcohol polyethoxylate (6.5)	6.5*	5.0*	21.5	-	5.0*
C <sub>14-15</sub> alcohol polyethoxylate (7)*	-	-	-	18.0	-
C <sub>12</sub> alkyl trimethylammonium chloride	1.2	0.6	-	-	0.6
Ditallowalkyl dimethyl ammonium chloride	-	-	-	3.6	-
C <sub>12-14</sub> alkyl dimethyl amine oxide	-	-	-	4.0	-

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$C_{12-14}$ fatty acid	13.0	10.0	-	-	7.7
Palm kernel fatty acid	-	-	-	-	3.3
Oleic acid	2.0	0.5	-	-	2.0
Citric acid (anhydrous)	4.0	4.0	-	-	4.0
Sodium diethylenetri-					
amine pentaacetate	0.3	0.3	-	-	0.3
Protease enzyme			As indicated		
Amylase enzyme (325 Am. U/g)	-	-	-	-	0.16
TEPA-E <sub>15-18</sub> **	1.5	2.0	-	1.5	2.0
Soil release compound****	-	-	-	-	2.5
Monoethanolamine	2.0	2.0	-	-	1.0
Sodium hydroxide	1.7	4.0	-	-	2.0
Potassium hydroxide	4.0	1.6	-	-	5.4
1,2 Propane diol	7.25	4.0	-	-	6.5
Ethanol	7.75	8.5	5.7	7.5	7.0
Boric acid			As indicated		
Sodium formate	1.0	1.0	1.6	1.2	1.0
Total calcium ion*** (mm/l)	9.65	9.65	0.25	0.25	9.65
Minors and water			Balance to 100		

Initial pH of 0.2% solution

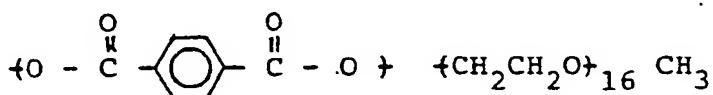
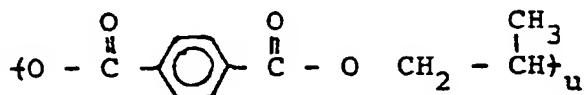
in distilled water at 20°C 7.5 7.5 7.2 7.2 7.5

\* Alcohol and monoethoxylated alcohol removed.

\*\* Tetraethylene pentamine ethoxylated with 15-18 moles (avg.) of ethylene oxide at each hydrogen site.

\*\*\* Includes estimated 0.25 millimoles of calcium ion per liter from enzyme slurry and formula water.

\*\*\*\* A compound having a range of copolymers of the formula:



in which about 20% by weight of the material has a value of  $u$  higher than 5 is dissolved at about 15% level in anhydrous ethanol; cooled to about 10°C; the insoluble portion (~ 20%) is filtered; and enough ethanol is distilled to reduce the ethanol level to within the level in the formula.

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The following proteases were added to the above Compositions B, C and D at a level to provide an initial activity of 0.015 Anson units per gram. Protease stability, in terms of percent retained activity, was then determined after storage of the compositions for the indicated number of weeks. The results were as follows.

<u>Composition</u>	<u>% Retained activity at 37.7°C</u>						
	<u>1 wk</u>	<u>2 wk</u>	<u>3 wk</u>	<u>4 wk</u>	<u>5 wk</u>	<u>6 wk</u>	<u>8 wk</u>
B + Alcalase*	13	0					
B + Maxatase**	15	0					
B + Protease A	10	0					
B + 1.25% boric acid + Alcalase	63	39	25	16	7	5	2
B + 1.25% boric acid + Maxatase	67	39	24	13	6	6	2
B + 1.25% boric acid + Protease A	98	75	53	50	33	30	18
C + Alcalase	94	88	76	70	60	66	
C + Protease A	100	91	80	71	61	63	
D + Alcalase	93	96	92	82	71	77	
D + Protease A	100	90	62	64	54	64	

\*Tradename of Novo Industries A.S.

\*\*Tradename of Gist-Brocades N.V.

<u>Composition</u>	<u>% Retained activity at 21.1°C</u>					
	<u>1 wk</u>	<u>2 wk</u>	<u>3 wk</u>	<u>4 wk</u>	<u>5 wk</u>	<u>6 wk</u>
B + Alcalase	89	85	85	95	85	89
B + Maxatase	92	94	92	100	100	100
B + Protease A	100	100	100	100	100	100
B + 1.25% boric acid + Alcalase	92	99	96	96	95	100

B + 1.25% boric acid + Maxatase	98	100	100	98	93	100
B + 1.25% boric acid + Protease A	100	100	100	97	100	100
C + Alcalase	100	100	100	99	93	100
C + Protease A	100	100	100	100	100	100
D + Alcalase	100	95	87	85	88	100
D + Protease A	100	100	100	100	100	100

The above results demonstrate that all three proteases quickly lost activity in Composition B at 37.7°C. The addition of 1.25% boric acid to Composition B improved the stability of all three proteases, but to a much greater degree for Protease

A. At 37.7°C, the stability of Protease A was comparable to Alcalase in Composition C, and slightly worse in Composition D. All three proteases were stable in composition B, C and D at 21.1°C.

The stability of Protease A and Maxatase in Compositions B with varying levels of boric acid was as follows.

Maxatase - % Retained Activity at 26.6°C							
Boric acid	0 wk	1 wk	2 wk	3 wk	4 wk	5 wk	6 wk
0%	100	98	93	92	95	90	83
0.25%	100	100	89	90	96	94	87
0.50%	100	100	100	99	95	95	88
0.75%	100	95	87	85	85	84	87
1.0%	100	99	84	84	92	95	88
1.25%	100	98	96	96	90	92	82
Protease A - % Retained Activity at 26.6°C							
Boric acid	0 wk	1 wk	2 wk	3 wk	4 wk	5 wk	6 wk
0%	100	100	90	87	89	87	82
0.25%	100	100	90	89	90	90	89
0.50%	100	97	95	95	93	92	92
0.75%	100	98	97	100	98	100	90
1.0%	100	100	98	100	98	100	93
1.25%	100	100	100	100	100	98	97

<u>Boric acid</u>	<u>Maxatase - % Retained Activity at 32.2°C</u>						
	<u>0 wk</u>	<u>1 wk</u>	<u>2 wk</u>	<u>3 wk</u>	<u>4 wk</u>	<u>5 wk</u>	<u>6 wk</u>
0%	100	69	53	40	40	32	20
0.25%	100	84	66	60	46	42	36
0.50%	100	86	74	69	60	54	45
0.75%	100	87	73	73	67	64	53
1.0%	100	93	79	80	70	68	61
1.25%	100	94	83	79	77	70	61

<u>Boric acid</u>	<u>Protease A - % Retained Activity at 32.2°C</u>						
	<u>0 wk</u>	<u>1 wk</u>	<u>2 wk</u>	<u>3 wk</u>	<u>4 wk</u>	<u>5 wk</u>	<u>6 wk</u>
0%	100	67	42	30	22	20	12
0.25%	100	85	70	72	60	52	45
0.50%	100	86	72	67	68	67	60
0.75%	100	97	79	90*	79	80	75
1.0%	100	97	92	90	92	90	79
1.25%	100	100	95	92	85	89	80

\*Apparently erroneous data.

<u>Boric acid</u>	<u>Maxatase - % Retained Activity at 37.7°C</u>						
	<u>0 wk</u>	<u>1 wk</u>	<u>2 wk</u>	<u>3 wk</u>	<u>4 wk</u>	<u>5 wk</u>	<u>6 wk</u>
0%	100	2.4	0.5	0.4	0.4	0.4	0.1
0.25%	100	12	3.6	1.2	0.7	0.8	0.4
0.50%	100	26	11	6	3	1	2
0.75%	100	34	15	8	3	2	1
1.0%	100	45	24	16	10	6	4
1.25%	100	46	29	20	14	7	5

<u>Boric acid</u>	<u>Protease A - % Retained Activity at 37.7°C</u>						
	<u>0 wk</u>	<u>1 wk</u>	<u>2 wk</u>	<u>3 wk</u>	<u>4 wk</u>	<u>5 wk</u>	<u>6 wk</u>
0%	100	1.6	0.8	0.3	0.2	0.3	0
0.25%	100	21	8	1.6	1	0.5	0.3
0.50%	100	40	19	11	8	3	2
0.75%	100	56	31	30	18	11	7
1.0%	100	67	48	40	35	17	15
1.25%	100	72	57	48	38	33	21

Protease A and Maxatase had comparable stability in Composition B without boric acid. The addition of boric acid improved the stability of both proteases, but to a much greater degree for Protease A, particularly at longer storage times and higher temperatures.

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In Composition C, Protease A, Alcalase and Maxatase had similar stability. The addition of boric acid improved the stability of Protease A, and decreased the stability of Alcalase and Maxatase, as demonstrated by the following results.

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		% Retained Activity at 37.7°C					
Protease A +	0 wk	1 wk	2 wk	3 wk	4 wk	5 wk	6 wk
0% boric acid	100	88	63	50	46	41	32
0.5% boric acid	100	90	93	87	80	77	76
1.25% boric acid	100	97	97	79	89	82	78
<u>Alcalase +</u>							
0% boric acid	100	93	66	36*	50	41	42
0.5% boric acid	100	81	56	54	30	23	17
1.25% boric acid	100	73	44	22	19	14	12
<u>Maxatase +</u>							
0% boric acid	100	86	72	22*	51	47	44
0.5% boric acid	100	83	63	43	35	31	23
1.25% boric acid	100	86	53	33	27	17	14

In Composition D, Alcalase and Maxatase were significantly more stable than Protease A. However, the addition of boric acid directionally improved the stability of Protease A but decreased the stability of Alcalase and Maxatase, as demonstrated by the following results.

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		% Retained Activity at 37.7°C						
Protease A +	0 wk	1 wk	2 wk	3 wk	4 wk	5 wk	6 wk	
0% boric acid	100	16	3	0.8	0.5	0.3	0.2	
0.5% boric acid	100	37	3	0.6	0.1	0.3	0.1	
1.25% boric acid	100	50	7	1	0.4	0	0.1	
<u>Alkalase +</u>								
0% boric acid	100	76	59	43	34	26	24	
0.5% boric acid	100	16	7	5	5	4	0.2	
1.25% boric acid	100	22	10	8	7	6	5	
<u>Maxatase +</u>								
0% boric acid	100	74	50	35	30	20	16	
0.5% boric acid	100	18	7	4	4	2	1	
1.25% boric acid	100	25	10	6	6	4	2	

The above results demonstrate that Protease A has a substantially different stability profile in combination with boric acid than does Alcalase or Maxatase.

In Composition B with boric acid, protease A and variants of Protease A in which the Gly at position 166 is replaced with Ser or Asn, the Gly at position 169 is replaced with Ser, or the Met at

position 222 is replaced with Phe, also exhibited improved stability versus Alcalase and the variant of Protease A in which the Met at position 222 is replaced with Gln, as demonstrated by the following results.

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		% Retained activity at 37.7°C					
0% Boric Acid +		0 wk	1 wk	2 wk	3 wk		
Alcalase		100	10	0.9	0.2		
Protease A		100	5	0.5	0		
Asn-166		100	21	9	-		
Gln-222		100	18	2	-		
Ser-169		100	4	0.7	-		
Phe-222		100	6	1	-		
Ser-166		100	18	1	-		
1.25% B.A. +		0 wk	1 wk	2 wk	3 wk	4 wk	5 wk
Alcalase		100	62	43	29	17	12
Protease A		100	77	73	53	48	40
Asn-166		100	78	70	68	51	46
Gln-222		100	93	36	6	3	2
Ser-169		100	49	47	37	33	26
Phe-222		100	-	-	52	40	32
Ser-166		100	77	83	77	66	60
							54

Compositions A and E of the present invention contain 0.75% of a slurry of Protease A, providing an activity of 0.015 Anson units per gram of composition, and 1.25% boric acid in place of water.

The invention herein can also be utilized in light-duty liquid detergent compositions, such as those described in U.S. Patent 3,634,266, Thiele et al, U.S. Patent 3,799,879, Francke et al, U.S. Patent 3,707,505, Maeda et al, U.S. Patent 4,316,824, Pancheri, and U.S. Patent 4,457,856, Mitchell et al, and in hard surface cleaning compositions, such as described in U.S. Patent 3,981,826, Munro, and U.S. Patent 3,985,668, Hartman.

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#### Claims

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1. A liquid detergent composition comprising, by weight:

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(a) from about 1% to about 75% of a synthetic detergent surfactant;

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(b) from about 0.01% to about 5% of the proteolytic enzyme characterized by the following amino acid sequence:

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1 Ala Gln Ser Val Pro Tyr Gly Val Ser Gln Ile Lys Ala Pro  
20  
Ala Leu His Ser Gln Gly Tyr Thr Gly Ser Asn Val Lys Val  
30 40  
Ala Val Ile Asp Ser Gly Ile Asp Ser Ser His Pro Asp Leu  
50  
Lys Val Ala Gly Gly Ala Ser Met Val Pro Ser Glu Thr Asn  
60 70  
Pro Phe Gln Asp Asn Asn Ser His Gly Thr His Val Ala Gly  
80  
Thr Val Ala Ala Leu Asn Asn Ser Ile Gly Val Leu Gly Val  
90  
Ala Pro Ser Ala Ser Leu Tyr Ala Val Lys Val Leu Gly Ala  
100 110  
Asp Gly Ser Gly Gln Tyr Ser Trp Ile Ile Asn Gly Ile Glu  
120  
Trp Ala Ile Ala Asn Asn Met Asp Val Ile Asn Met Ser Leu  
130 140  
Gly Gly Pro Ser Gly Ser Ala Ala Leu Lys Ala Ala Val Asp  
150  
Lys Ala Val Ala Ser Gly Val Val Val Ala Ala Ala Gly  
160  
Asn Glu Gly Thr Ser Gly Ser Ser Ser Thr Val Gly Tyr Pro  
170 180  
Gly Lys Tyr Pro Ser Val Ile Ala Val Gly Ala Val Asp Ser  
190  
Ser Asn Gln Arg Ala Ser Phe Ser Ser Val Gly Pro Glu Leu

200

210

Asp Val Met Ala Pro Gly Val Ser Ile Gln Ser Thr Leu Pro  
 220

Gly Asn Lys Tyr Gly Ala Tyr Asn Gly Thr Ser Met Ala Ser  
 230

Pro His Val Ala Gly Ala Ala Ala Leu Ile Leu Ser Lys His  
 240

250

Pro Asn Trp Thr Asn Thr Gln Val Arg Ser Ser Leu Glu Asn  
 260

Thr Thr Thr Lys Leu Gly Asp Ser Phe Tyr Tyr Gly Lys Gly  
 270

275

Leu Ile Asn Val Gln Ala Ala Ala Gln;

or wherein the Gly at position 166 is replaced  
 20 with Ser or Asn, the Gly at position 169 is  
 replaced with Ser, or the Met at position 222 is  
 replaced with Phe;

(c) from about 0.1% to about 10% of boric acid  
 25 or a boron compound capable of forming boric  
 acid in the composition;

(d) from about 0.01 to about 50 millimoles of  
 30 calcium ion per liter of composition; and

(e) from about 10% to about 95% of water.

2. A composition according to Claim 1 comprising  
 35 from about 5% to about 50% of synthetic  
 detergent surfactant.

3. A composition according to Claim 2 comprising  
 40 an anionic synthetic surfactant.

4. A composition according to Claim 3 comprising  
 45 from about 15% to about 30% of an anionic  
 surfactant which comprises a  $C_{10}$ - $C_{18}$  alkyl sulfate, a  $C_{10}$ - $C_{18}$  alkyl ethoxy sulfate containing an  
 average of up to about 4 moles of ethylene oxide  
 per mole of alkyl sulfate, a  $C_{10}$ - $C_{12}$  linear alkyl-  
 benzene sulfonate, or mixtures thereof.

5. A composition according to Claim 4 comprising

20 ing from about 30% to about 50% of a mixture  
 of an anionic synthetic surfactant and a deter-  
 gency builder, said composition having an initial  
 pH of from about 7.0 to about 8.5 at a concentra-  
 tion of 0.2% in water at 20°C.

25 6. A composition according to Claim 5 comprising  
 from about 5% to about 20% of a saturated  
 fatty acid containing from about 10 to about 14  
 carbon atoms.

30 7. A composition according to Claim 5 comprising  
 from about 1% to about 20% of a water-  
 soluble polycarboxylate builder.

35 8. A composition according to Claim 5 comprising  
 from about 0.5% to about 3% of boric acid.

40 9. A composition according to Claim 8 further  
 comprising from about 1% to about 15% of a  
 polyol containing from 2 to 6 carbon atoms and  
 from 2 to 6 hydroxy groups.

45 10. A composition according to Claim 9 wherein  
 the polyol comprises 1,2 propane diol.

11. A composition according to Claim 8 further  
 comprising from about 0.4% to about 1.5% of a  
 water-soluble formate.

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